Influence of a gel emulsion containing microalgal oil and a blackthorn (*Prunus spinosa* L.) branch extract on the antioxidant capacity and acceptability of reduced fat beef patties

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ABSTRACT

The addition of an extract from blackthorn branches (*Prunus spinosa* L.) to a gel emulsion system containing microalgal oil was examined in order to obtain a functional ingredient (APG), suitable to be used as fat replacer in beef patties. Catechins were the major flavonoids found by HPLC analysis in the *Prunus spinosa* L. extract. Comparing gel emulsions with (APG) and without (AG) the extract, antioxidant capacity increased as a result of the extract addition. Beef patties containing APG as fat replacer (modified patties), halved the fat content, doubled their antioxidant activity and DHA content and improved their stability against oxidation (peroxide values were reduced more than two-fold) as compared to control patties. Moreover, instrumental color remained unchanged and their sensory acceptability was confirmed by an hedonic test.

Key words: Blackthorn, Extract, Omega-3, Antioxidant, Gel, Fat replacer

1. Introduction

Fruits, spices, herbs and essential oils, among other plant-derived options have been explored as potential sources of antioxidant compounds. These natural plant derivatives have been used for the development of healthier meat products (Jiang & Xiong, 2016) and it has been even pointed out that their presence may contribute to decrease the risk of several degenerative diseases (Hygreeva, Pandey, & Radhakrishna, 2014).

Another important consideration regarding the use of natural antioxidants in meat products is their impact on sensory aspects. Effectively, although this aspect is not always evaluated, it is crucial that antioxidants enriched products present adequate sensory properties for the consumer acceptability.

Adding antioxidants can be particularly necessary when reformulation of products implies increasing their content in omega-3 fatty acids as a healthy strategy (Navas-Carretero et al., 2015). In the particular case of reformulated beef patties enriched in omega-3 and natural antioxidants, not many papers have been found in the literature to illustrate this issue. Valencia, O'Grady, Ansorena, Astiasaran, & Kerry (2008) reported the protective effect of green tea catechins against lipid oxidation in omega-3 enriched fresh pork sausages. The addition of olive leaf extract also conferred a protective effect in pork patties with increased levels of omega-3 content (Botsoglou, Govaris, Ambrosiadis, Fletouris, & Papageorgiou, 2014).

Prunus spinosa L. (blackthorn) is a shrub native to Europe, Western Asia and Northwest Africa. Its astringent fruits have been reported to have a high antioxidant activity (Ruiz-Rodríguez et al., 2014). Moreover, Pinacho, Cavero, Astiasarán, Ansorena, & Calvo

(2015) showed the antioxidant activity of branches of a pure ethanol extract of *Prunus spinosa* L. In fact, values reported for total phenolic compounds, total flavonoid content and antioxidant activity were higher for this part of the plant than for the fruits. Thus, the possibility of using wild blackthorn branches (*Prunus spinosa* L.) as source of antioxidants for enhancing the functionality of meat products merits investigation.

The aim of this work was to assess the viability of a functional ingredient (a gel emulsion) rich in long-chain omega-3 fatty acids and natural antioxidants from fresh branches of *Prunus spinosa* L. as a fat replacer in raw and cooked beef patties. Nutritional, physicochemical and sensory aspects were taken into consideration.

2. Material and methods

2.1. Materials

Chemicals used: fatty acid methyl esters, NaCl, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid 97%), ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), DPPH (2,2-diphenyl-1-picrylhydrazyl) and gallic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Boron trifluoride, methanol and heptane were obtained from Merck (Whitehouse Station, NJ, USA). Folin-Ciocalteu reagent was supplied by Panreac (Barcelona, Spain).

For the gel emulsion, DHASCO® microalgal oil (40% docosahexaenoic acid) obtained from microalga *Crypthecodinium cohnii* was provided by Martek Biosciences Corporation, Columbia, USA. Kappa-carrageenan was provided by Cargill (San Sebastián, Spain) and polysorbate 80 was obtained from Sigma-Aldrich Chemical Co.

(MO, USA). For the beef patties, fresh lean minced beef and fresh minced pork back fat were obtained from a local meat market.

2.2. Blackthorn branch extract manufacture

Fresh branches of *Prunus spinosa* L. were collected in Navarra in 2016. The plant was identified by Dra. R.Y. Cavero, air-dried, ground into fine powder (180 mesh), and deposited in the herbarium of the Department of Environmental Biology, Faculty of Science, University of Navarra, Spain (Voucher: PAMP 21961). The extraction was carried out by cold maceration (4ºC) using 50% ethanol in a closed container several times. The extract was dried under reduced pressure at 30 °C in a rotary evaporator and then were lyophilised (Virtis BT3-SL, NY, USA) and stored in glass vial at −40 °C until tested and analysed.

2.3. HPLC- DAD analysis instrumentation

The hydroalcoholic extract from branches of *Prunus spinosa* L. was analysed by HPLC using a Waters (Milford, Massachusetts, USA) 600E multi-solvent delivery system, a Waters U6K sampler and a Waters 991 photodiode-array detector. The chromatographic conditions were the same as those applied by Pinacho et al. (2015). The identity of polyphenols was ascertained using data from HPLC/DAD and by comparison of spectral data with results from Pinacho et al. (2015).

2.4. Gel emulsion manufacture

Two different types of gel emulsions were prepared: Algal Gel (AG) and Algal *Prunus* Gel (APG). Both gel emulsions contained 1 % of microalgal oil, 3 % of carrageenan and 96 % of water were made following a standardized procedure developed by Alejandre, Passarini, Astiasarán, & Ansorena (2017). APG also contained 0.8% of *Prunus spinosa* L.

extract. This amount corresponded to the maximum concentration that allowed an adequate polymerization of the carrageenan during manufacture of the gel emulsion.

Both gels (AG and APG) were prepared according to the method described by Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasaran (2014). Part of the gel emulsions were lyophilized (Vitis BT3-SL, NY, USA) and stored in glass vials at -20º C until tested and analyzed. Two different replicates were prepared per each type of gel emulsions (AG and APG).

2.5. Determination of antioxidant activity and total phenolic compounds (TPC) of the *P. spinosa* extract and the gel emulsions

ABTS, DPPH and TPC were applied to determine the antioxidant activity and the content of phenolic compounds of the *P. spinosa* L. extract and also of the gel emulsions. In all cases, lyophilized samples were used.

ABTS and DPPH methods were carried out following the procedure described in Garcia-Herreros, Garcia-Iniguez, Astiasaran, & Ansorena (2010) using different dilutions depending on the sample (*P. spinosa* extract, AG or APG). Results were expressed as µg Trolox equivalents/ mg lyophilized sample.

TPC were determined spectrophotometrically following the Folin-Ciocalteu colorimetric method described in Garcia-Hereros et al. (2010). The amount of total phenolic compounds was expressed in µg gallic acid/mg lyophilized sample. Determinations were performed in triplicate.

2.6 Incorporation of the gel emulsions to beef patties

2. 6.1 Preparation of beef patties

Two different formulations of beef patties were manufactured in a pilot plant (2 kg batches). Two replicates of each formulation were carried out, on different days. For

the Control formulation (C) fresh lean minced beef (80%) and minced pork back fat (20%) were used. In the second formulation (M), the pork back fat was totally replaced by the Algal *Prunus* Gel (APG), freshly prepared. Both formulations also included the following common ingredients per kilogram of minced beef meat: 8 g salt, 5 g red pepper, 4 g dehydrated onion, 2 g garlic powder and 1.5 g black pepper. The processing of beef patties (raw and cooked) was carried out following the procedure described in Alejandre et al. (2017). Patties were aerobically packaged in plastic bags and stored at −20°C until analysis. The sensory evaluation of cooked products was carried out just after cooking (day 0).

2.6.2 Nutritional analysis of beef patties

Proximate composition analyses were performed on raw and cooked patties of each replicate and formulation, with three measurements per sample. Quantification of moisture, protein, ash and fat was done using official methods (AOAC, 2002). Extraction of lipids was carried out using fresh sample (120 g) and a chloroform: methanol mixture (2:1), according to Folch, Lees, & Stanley, (1957). Fatty acid profile was determined in the lipid extracts by gas chromatography. Detailed description of derivatization, chromatographic conditions, identification and quantification of fatty acid methyl esters is described in Alejandre et al. (2017).

2.6.3 Antioxidant activity of beef patties (ABTS method)

ABTS method was carried out following the procedure described in Garcia-Herreros et al. (2010) on control and modified patties, in raw and cooked samples. Lyophilized samples were used.

2.6.4 Lipid oxidation analysis of beef patties

In order to assess the oxidation status of the beef patties, peroxides were measured. Peroxide Index (PI) was analysed at 510 nm following the method of Shanta & Decker (1994) with modifications. Results were expressed as mmol ROOH / kg product. Four measurements were done per sample.

2.7. Instrumental color

The color of the gel (APG), the beef ground meat and the control and modified raw patties was measured using a colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan). Six measurements were done in each sample. The gel emulsion was cut in cylinders (D=2.8 cm and h=1cm). For ground beef and patties, a homogenized ground mixture of each sample was taken. The following parameters were determined: lightness (L*), redness (a* ± red-green), and yellowness (b* ± yellow-blue). Color coordinates were obtained using the CIE L*a*b* system, angle 10°, illuminant D65.

2.8. Sensory evaluation

A hedonic test (Anzaldua-Morales, 1994) was performed to evaluate the overall acceptability of the cooked patties. 26 non-trained panelists participated in the study. This study was carried out in two sessions. In each session, each panelist scored two samples: control and modified patties (C and M), with a 9-point scale. The scores ranged from 1 to 9 (9. like extremely; 8. like too much; 7. like considerably; 6. like slightly; 5. not like no dislike; 4. dislike slightly; 3. dislike considerably; 2. dislike too much; 1. dislike extremely). Each point marked was converted to a numerical value (from 0 to 9) assigned to the descriptive terms of the questionnaire so that further statistical analyses of data could be performed. Conditions of the test were the same as are described in Alejandre et al. (2017).

2.9. Statistical analysis

The statistical analysis of data was done using the STATA/IC 12.1 program (StataCorp LP, Texas, USA). The whole experiment (elaboration of the gel emulsions and application to the beef patties) was carried out in duplicate. For antioxidant activity, total phenolic compounds and color parameters, one-way analysis of variance (ANOVA) was performed to evaluate the statistical significance ($P < 0.05$) among formulations. Formulation was assigned as fixed effect and replication as random effect. Multiple comparisons of means were done using Bonferroni Post Hoc procedure to evaluate the statistical significance ($P \leq 0.05$) among formulations. Antioxidant activity and fatty acid profile of the beef patties were analysed using 2x2 factorial ANOVA as a function of formulation and cooking process. Replication was considered as random effect. The values in the tables were given in terms of mean values and standard error of the mean (SEM). With regard to sensory analysis, numerical values obtained in the sensory test were evaluated by ANOVA. Formulation was considered the fixed effect whereas evaluation session and panelists were considered random effects.

3. Results and discussion

3.1 Characterization of the extract and gels

The characterization of the antioxidant activity of the hydroalcoholic extract from branches of *P. spinosa* L. was performed by three spectrophotometric techniques (ABTS, DPPH and TPC) (table 1). High values of antioxidant activity were observed both by the ABTS test (227.4 µg TE/mg lyophilized extract) and the DPPH method (104.4 µg TE/mg lyophilized extract). TPC value was 154.5 µg GA/mg lyophilized extract. According to the chromatographic analysis (Figure S1, Supplementary Material), the

two major compounds identified by HPLC in this extract were *Ent*-(epi)-afzalechin (2α→*O*→7, 4α→8) catechin (peak 1) and *Ent*-(epi)-afzalechin (2α→*O*→7, 4α→8)-(epi)-catechin (peak 2), which were also present in methanolic and water prepared extracts of blackthorn branches (Pinacho et al., 2015). Values of antioxidant activity and content of phenolic compounds were within the range of data observed in previous plant-derived extracts used by our group as antioxidants in meat products: *Borago officinalis* (García Iñiguez de Ciriano et al., 2009) and *Melissa officinalis* (García Iñiguez de Ciriano et al., 2010).

The previously described extract was incorporated to a low-energy gel emulsion (0.8%) aiming to obtain a functional ingredient. The addition of the extract to the gel emulsion, which also contained 1% microalgal oil and 3% carrageenan, resulted in a significant increment of its antioxidant activity. According to the results shown in table 1, the antioxidant activity of the Algal Gel emulsion (AG) was very low, whereas when the *P. spinosa* extract was added (APG), significantly higher values were found. Per each formulation, no significant differences ($P>0.05$) between replicates were found. Once the antioxidant activity of the APG was demonstrated, this functional ingredient was used as total fat replacer in beef patties.

3.2. Application in patties

The nutritional quality of the modified patties, which included the APG, was studied by the analysis of the proximate composition, both in raw and cooked samples. For control cooked patties, values for moisture, fat, protein and ash were 61.12%, 10.75%, 23.12% and 1.87%, respectively. Modified patties presented changes in their composition, especially in the fat content. In addition to the already low fat content of

199 control cooked patties (10.75%), modified patties showed a significant fat reduction
200 (52%) in their composition, showing a final value of 5.20% after cooking. Protein was
201 21.4%, moisture was 67.6% and ash was 1.93%. Regarding the lipid composition of the
202 final modified cooked product, the incorporation of the APG contributed to a
203 significant increment of DHA (29.2mg/100g, whereas control showed 14.4mg/100g), a
204 significant reduction of the total saturated fraction (53% reduction), a relevant
205 decrease in the omega-6/omega-3 ratio (10, as compared to 30 in the control), among
206 other interesting data (Table S1. Supplementary material). These results confirmed
207 those found in a previous work (Alejandre et al., 2017), where an emulsion containing
208 microalgal oil without extract, gave rise to a significant fat reduction and to an
209 improved lipid profile of the developed patties.

210 Besides the nutritional benefits of the modified patties, the effect of the addition of
211 the extract on the modified patties was measured through ABTS test. Higher values for
212 ABTS were noticed in modified patties (M), as compared to patties without the extract
213 (C) (Table 1). For control patties, ABTS values were 0.29 and 0.30 µg Trolox
214 equivalents/mg lyophilized sample (raw and cooked, respectively), while for modified
215 patties, ABTS values were 0.65 and 0.56 µg Trolox equivalents/mg lyophilized sample,
216 for raw and cooked patties, respectively. These data lead to conclude about the
217 thermal stability of the extract, as it remains active despite the heating treatment.

218 Consequently, it could be hypothesised that the higher antioxidant activity shown by
219 modified patties could contribute to control their lipid oxidation. In order to verify this
220 hypothesis, lipid oxidation was monitored before and after the cooking process of the
221 patties, through the evaluation of the presence of primary (lipid hydroperoxides)

222 oxidation products. For control patties, values of peroxides were 0.42 and 0.27 mmol
223 ROOH/ kg product in raw and cooked patties, respectively. For modified patties, 0.18
224 in raw and 0.04 mmol ROOH/ kg product were found in cooked patties. It could be
225 noticed that in both raw and cooked samples, modified patties showed lower lipid
226 oxidation values than controls (P<0.05). The addition of *P. spinosa* extract may have
227 contributed to protect the reformulated patties against lipid oxidation. Also, the lower
228 fat content of the modified patties may explain this reduced lipid oxidation, as
229 previously stated by Alejandre et al. (2017).

230 On the other hand, color is considered an important factor that could impact
231 purchasing decisions of beef (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017).
232 Therefore, the incorporation of the gel into the patties should not generate a
233 substantial modification of the visual aspect of the new formulations. The color of APG
234 was evaluated and compared to data obtained from ground beef meat (table 2)
235 Additionally, color of raw beef patties with and without the gel emulsion (modified and
236 control patties) was also measured. Color of beef and APG showed similar values in
237 lightness (L*), though APG had higher yellowness (b*) and lower redness (a*) than
238 beef meat. Despite the difference, when APG was added to the beef patties, no
239 significant changes in these parameters were found between C and M Raw patties
240 (P>0.05). Holman et al. (2017) reported redness parameter (a*) as the most indicative
241 of beef color acceptability. Both patties (C and M) showed a redness value similar to
242 that obtained for the beef meat (P>0.05). These results indicate that APG, which was
243 chopped and added to the modified patties, was perfectly integrated into the final
244 meat matrix.

The replacement of animal fat in modified patties could have an important effect in the consumer acceptability of the new products. Moreover, the incorporation of the plant extracts at non-adequate doses might also lead to unpleasant sensory notes in beef patties (Barriuso et al., 2015). Therefore, both control and modified products were subjected to sensory evaluation by means of a hedonic test. The mean "general acceptability" score received by control patties was 6.88, whereas modified patties had 6.58 points in the hedonic scale, showing no statistical differences ($P>0.05$) in comparison to the control product. No significant differences ($P>0.05$) were found between sessions. These results allowed us to conclude about the positive evaluation of consumers on the acceptability of the new product, which was similar to that reported for the traditional one.

4. Conclusion

In summary, the natural extract from blackthorn branches (*Prunus spinosa* L.) was particularly rich in catechins, which provided high antioxidant activity to the gel emulsion containing the extract (APG). This gel was incorporated as a functional ingredient in beef patties for the development of a healthier formulation. The modified patties improved their nutritional quality (51% of fat reduction and relevant long chain omega-3 fatty acids content) and increased their antioxidant activity as compared to control patties, giving rise to a lower lipid oxidation of the modified patties than the control ones. Also, the addition of the extract did not affect the color and the overall sensory acceptability of the modified patties. Further research into the bioaccessibility of bioactive compounds contained in blackthorn branches is necessary in order to ascertain their protective effects and potential benefits to human health.

5. Acknowledgments

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TABLE CAPTIONS

Table 1. . Antioxidant activity and total phenolic compounds content of *Prunus spinosa* extract, the gel emulsions; Algal Gel (AG) and Algal Prunus Gel (APG) and the beef patties: Control and Modified before and after cooking.

Table 2. Color CIE L* a* b* evaluation of Algal *Prunus* Gel (APG), beef meat and control (C) and modified (M) raw beef patties.

Table 1. Antioxidant activity and total phenolic compounds content of *Prunus spinosa* extract, the gelled emulsions Algal Gel (AG) and Algal Prunus Gel (APG) and the beef patties: Control and Modified before and after cooking.

	Extract	**Gelled emulsions**			**Beef patties**						
	P. spinosa	AG	APG	*P value*	C Raw	C cooked	M Raw	M Cooked	*P values*[1]		
									Formulation	Cooking	Interaction
ABTS	227.4 (0.3)	0.2 (0.0)a	30.8 (0.6)b	0.000	0.29 (0.04)a	0.30 (0.01)a	0.65 (0.04)b	0.56 (0.05)b	0.001	ns	0.04
DPPH	104.4 (2.9)	0.1 (0.0)a	9.5 (0.5)b	0.000	-	-	-	-	-	-	-
TPC	154.5 (1.8)	0.7 (0.0)a	37.6 (0.5)b	0.000	-	-	-	-	-	-	-

AG: Algal gel; APG: Algal Prunus gel; C: Control; M: Modified; ns: no significant; - : data not measured. ABTS are expressed in µg Trolox equivalents/mg lyophilized sample, DPPH in µg Trolox/mg lyophilized sample and TPC in µg gallic acid/mg lyophilized sample. Standard errors of the mean (SEM) appear in parentheses. [1]*Results from 2x2 factorial ANOVA. For each parameter, different letters (a,b) indicate significant differences (P< 0.05) based on post hoc Bonferroni test. No significant differences (P>0.05) were found between replicates.*

Table 2. Color CIE L* a* b* evaluation of Algal *Prunus* Gel (APG), beef meat and control (C) and modified (M) raw beef patties.

	APG	**Beef**	**C Raw**	**M Raw**	***P* value**
L*	48.8 (0.12)b	48.4 (0.09)b	43.4 (0.6)a	42.6 (0.3)a	0.000
a*	14.9 (0.05)a	19.0 (0.02)b	20.4 (0.4)b	18.3 (0.4)b	0.000
b*	26.1 (0.08)c	10.0 (0.05)a	16.6 (0.4)b	17.2 (0.3)b	0.000

Standard error of the mean (SEM) appear in parentheses. For each parameter, different letters in the same row (a, b, c) indicate significant differences (P< 0.05) based on post hoc Bonferroni test. No significant differences (P>0.05) were found between replicates.

SUPPLEMENTARY MATERIAL

"Influence of a gel emulsion containing microalgal oil and a blackthorn (*Prunus spinosa* L.) branch extract on the antioxidant capacity and acceptability of reduced fat beef patties"

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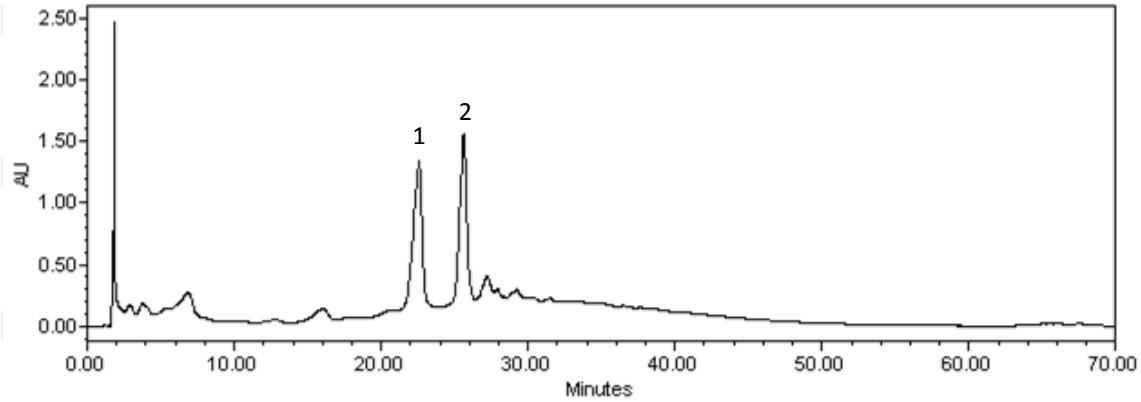

Figure S1. HPLC profile of the *Prunus spinosa* L. hydroalcoholic extract. Peaks 1 and 2 correspond to Ent-(epi)-afzalechin (2α→O→7, 4α→8) catechin and Ent-(epi)-afzalechin (2α→O→7, 4α→8)-(epi)-catechin, respectively.

SUPPLEMENTARY MATERIAL

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Table S1. Fatty acid profile of the control (C) and modified (M) beef patties expressed in mg fatty acid per 100 g of product.

	Conditions				SEM	*P* values[1]		
	C Raw	C Cooked	M Raw	M Cooked		Formulation	Cooking	Interaction
Caprilic C8:0	1.6	2.3	0.9	1.4	0.1	***	***	ns
Capric C10:0	5.1	6.1	2.4	2.8	0.4	***	ns	ns
Lauric C12:0	5.6aA	7.7aB	4.3aA	4.7bA	0.4	***	**	*
Myristic C14:0	220.6aB	230.4bB	142.0aA	158.6bA	0.9	***	***	**
Palmitic C16:0	2694	2856	1377	1525	172	***	***	ns
***t*-palmitoleic C16:1 9t**	12.0	14.1	8.9	10.0	0.7	***	ns	ns
Palmitoleic C16:1	309.5aB	320.7bB	179.7aA	201.4bA	16.3	***	***	**
Stearic C18:0	1555aB	1652bB	798.2aA	860.3bA	101	***	***	**
Elaidic C18:1 9t	31.8	23.1	15.5	11.4	2.1	***	***	ns
Oleic C18:1 (ω-9)	4032	4189	1788	1960	290	***	***	ns
c- Vaccenic C18:1 11c	188.5aB	188.1aB	12.4aA	23.2bA	22.1	***	ns	*
Linoleic C18:2Δ9c,12c (ω -6)	916.4	977.1	271.7	327.7	84.0	***	***	ns
Arachidic C20:0	9.1bB	8.7aB	3.4aA	4.4bA	0.7	***	***	***
Eicosenoic C20:1 (ω-9)	0.5	0.5	0.5	0.6	0.1	ns	ns	ns
α-linolenic C18:3 (ω-3)	15.8c	19.2	3.9	7.2	1.6	***	***	ns
Brasidic C22:1 13t	4.7aB	6.3bB	2.4aA	2.5bA	0.5	***	***	***
Erucic C22:1	6.3	7.2	1.2	1.6	0.9	***	ns	ns
Arachidonic C20:4 (ω-6)	62.3	77.0	35.6	52.4	4.2	***	***	ns
Eicosapentaenoic C20:5 (ω-3) (EPA)	2.1bB	1.4aA	0.8aA	1.3bA	0.1	***	ns	***
Docosahexaenoic C22:6 (ω-3) (DHA)	11.4aA	14.4bA	27.5aB	29.2bB	1.8	***	***	***
SFA	4481	4759	2329	2557	283	***	***	ns
MUFA	4535	4715	1998	2185	326	***	***	ns
PUFA	1016	1088	338	419	88	***	***	ns
EPA+DHA	13.5aA	15.7bA	28.3aB	30.2bB	1.8	***	***	***
Omega-3	29.3	34.9	32.2	37.8	1.0	*	**	ns
Omega-6	978.8	1054	309.0	380.1	87.3	***	***	ns
Omega-6/omega-3	33.4bB	30.2aB	9.6aA	10.1aA	2.9	***	ns	*
PUFA/SFA	0.20aB	0.20aB	0.14aA	0.13aA	0.01	***	ns	***
(PUFA+MUFA)/SFA	1.2	1.2	1.0	1.0	0.03	***	ns	ns
Trans	45.7	39.3	27.9	23.9	3.14	***	***	ns

*SEM: Standard error of the mean. [1]Results from 2x2 factorial ANOVA. Levels of significance: *: P<0.05; **: P<0.01; ***: P<0.001. ns: not significant based on post hoc Bonferroni test. Different small letters (a,b) indicate significant differences (P<0.05) between raw and cooked patties within same formulation (control or modified) Different capital letters (A,B) indicate significant differences (P<0.05) between control and modified patties within same cooking (raw or cooked).No significant differences (P>0.05) were found between replicates.*